Studies on Fungi Exposed to Space Irradiation

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Abstract. The cellulolytic fungus Chaetomium globosum Kunze et Fries was exposed to spaceflight parameters on board the Apollo 16 Command Module. Flight phenotypic isolates lost pigment forming capabilities. Ultraviolet light irradiation of C. globosum during deployment of the MEED hardware caused a reduction of perithecial development. In addition the returned spaceflight phenotypes demonstrated variation in α-amylase enzyme production.

Introduction

Selected fungal species were placed in the Microbial Ecology Evaluation Device (MEED) of Apollo 16 and were exposed to various quantitative and qualitative spaceflight parameters then returned to earth for various postflight analyses (Volz, 1972). The MEED hardware passed rigid weight and size requirements yet maintained full experimental capabilities (Taylor, 1970). Ascospores of C. globosum were housed in MEED cuvettes and maintained in a controlled state that prohibited spore germination yet maintained survival qualities until exposure to ultraviolet light irradiations in space. The passive life support system for the spores provided a stable environment before, during, and after deployment of the MEED package in space. Technical design requirements for the microbial cuvettes maintained a thermal environment of 20 C $\pm$ 3 C throughout the Apollo flight and during the 10 minute + 7 second MEED deployment on the transearth Extra Vehicular Activity (Volz, 1972).

Select MEED phenotypes of C. globosum from Apollo 16 were isolated for the current threefold postflight study. The first segment consisted of a growth dynamic study to identify changes in dry weight. The second segment consisted of a colony morphology investigation to qualitatively identify extracellular α-amylase of the select phenotypes.

Chaetomium globosum is a valuable organism for study in space related experiments. Both filamentous fungi C. globosum and T. terrestre provided an excellent model for studies in nutritional requirements (Volz, 1973b), chromosome configurations (Hsu et al., 1973a, 1973b; Jerger and Volz, 1977), cell growth dynamics (Volz and Dublin, 1973; Volz and Jerger, 1973) in addition to biochemical studies (Sawyer et al., 1975a, 1975b), radiation exposure (Volz et al., 1974), and medical studies (Hsu and Volz, 1975; Hiebel and Volz, 1977; Veselenak and Volz, 1977).

Chaetomium globosum is known to be a spaceship and spacesuit contaminant (Henney, 1971, 1972; Henney and Arredondo, 1973; Truby, 1968; Volz, 1973a), thus any change incurred by the organism during spaceflight may affect the well being of the astronauts in space. Prior to space studies C. globosum was considered of economic

significance in military and industrial operations due to the ability to degrade cellulose material (Moore-Landecker, 1972).

Literature Review

α-Amylase is commonly found in most organisms of the plant and animal kingdoms. Microorganisms utilize amylases to hydrolyze carbohydrate substrates into oligosaccharides or sugars (Whitaker, 1972).

Chaetomium globosum is a cellulolytic ascomycete. Extracellular enzymes produced by this organism destroy fibers, paper, and other materials composed of cellulose. Damage caused by the Chaetomiales each year is extensive. The organisms are easily recognized by their characteristic perithecia produced superficially without a stroma that possess numerous long curly perithecial hairs. The gelatinous walls deliquesce before spore maturity. Ascospores formed in asci are lemon shaped and dark brown in color, thick walled, and contain oil droplets in the protoplasm (Hsu, 1973).

Chaetomium globosum has septate hyphae which with the MEED phenotypes have different growth patterns. Hyphal growth occurs expontentially during the early stages of colony development. During the latter stages of colony development, however, growth in the center of the colony declines while the marginal hyphae maintain their linear rate of growth (Plomley, 1959).

Chaetomium species have been used in experiments with radiation sources. Dickson (1932, 1933) exposed Chaetomium colonies growing on malt agar and ascospores of several species to X-rays for 50 minutes at a distance of 26 cm. Changes in color and amount of mycelium were induced in colonies arising from subcultures of irradiated species. Chaetomium globosum produced maximum mutations after irradiation at 280 nm (McAulay and Ford, 1947). Ultraviolet radiation at 254 nm has a marked lethal effect on C. globosum (Hsu, 1973). In the MEED spaceflight study it was found that high energy multicharged particles (HZE) in space caused breaks in chromosomes of living organisms (Benton and Henke, 1973). Studies on UV absorption of DNA indicated that DNA absorption peaks were at 240 nm. Absorption of UV light by proteins occurs at 275 nm, while the relative germacidal effectiveness of UV peaks is 260 nm (Taylor, 1970). Phenotype counts and viability rates of C. globosum exposed to spaceflight parameters including known levels of UV light were examined (Volz, 1973b). The organism degrades spacesuit material and Apollo Extravehicular Modular Unit (EMU) fabrics (Volz and Jerger, 1973). The material is utilized as a direct food source. A spacesuit degraded by the fungus has a direct effect on the health and safety of astronauts in space.

Materials and Methods

Chaetomium globosum perithecia were harvested 14 days before launch from colonies grown on corn meal agar. Ascospores were removed from the perithecia with a sterile glass rod and placed in quartz cuvettes for UV exposure in the MEED spaceflight hardware (Volz, 1972). After splashdown of Apollo 16 and the return of the MEED to the laboratory, postflight studies were initiated on the recovered ascospores and phenotype colonies (Volz, 1974). Phenotypes of C. globosum were selected by perimeter growth rates, colony pigmentation, reverse colony color, perithecial density, and genetic restriction of colony

compatibility. Stock colonies were maintained on Sabouraud maltose agar.

Select phenotypes of C. globosum were grown in 250 ml Erlenmeyer shake culture flasks containing 75 ml of Omeliansky's medium. The cultures were harvested after one week growth. Known concentrations (2.5×10^5 hyphal units/ml) of blended phenotype mycelium were innoculated into new culture flasks and the culture procedure repeated. The cultures grew for one week at 25 C, harvested, and weighed using preweighed Whatman #1 filter paper. Select phenotype mycelial weights were then compared. Colony morphology was studied as cultures matured, and the distinctive features were noted.

Select phenotypes were grown 10 days in Omeliansky's liquid medium on shakers for the α-amylase studies. Harvested phenotypes were homogenized in a sterile blender one minute. A known concentration of fungal suspension of each phenotype was reinoculated in 50 ml Omeliansky's liquid medium in 250 ml shaker flasks in replicates of three. α-Amylase was analyzed using the RBB starch method (Hall et al., 1970). To each of 3 test tubes (two duplicate samples and a blank), 0.3 ml of the flask medium was pipetted. The medium contained select actively growing cultures of C. globosum. Just prior to use, the substrate suspension was prepared. The substrate was composed of 2.0 ml RBB starch, in 100 ml substrate buffer. The substrate buffer consisted of 2.76 g $NaH_2PO_3 \cdot H_2O$ and 2.93 g NaCl, adjusted for pH 7.0 with NaOH, and brought to a final volume of 1 liter. RBB starch used in this study was amylose asure B grade. The samples and substrate were preincubated in a 37 C water bath 15 min. The substrate was swirled to obtain a homogenous suspension while remaining in the water bath. Immediately, 2.7 ml of RBB starch substrate was added to the first sample tube, swirled to mix. The mixed substrate was pipetted into the remaining sample tubes. After 15 min the enzymatic activity of the first tube was terminated by the addition of 1.2 ml 18% aqueous acetic acid. Enzymatic activity in the remaining sample tubes was stopped with 18% acetic acid using the same sequence and time interval established when the substrate was added. The blank contained 0.3 ml sample, 2.7 ml substrate buffer without RBB starch, and 1.2 ml 18% acetic acid. After the enzymatic action was terminated, samples and blanks were well mixed and filtered through Whatman #1 filter paper. The supernatant was read at 595 nm in a Bauch & Lomb spectronic 20 spectrophotometer, using quartz cuvettes. The RBB starch filtrate was read, the absorbency relative to water of an aqueous solution of 0.1 M $CuSO_4$ was determined in duplicate.

Results

During the transearth Extra Vehicular Activity of Apollo 16 on April 25, 1972, the MEED hardware was deployed. The exposure levels received by each of the selected phenotypes for the current study are presented in Table I.

The wet flight cuvettes, housing cells in water, exposed the cells to full solar irradiation. Wet ascospores produced low viability rates for C. globosum. The dry cuvettes vented to space atmosphere and unvented cuvettes exposed the cells to full solar irradiation. Cell viabilities remained high in dry space flight cuvettes

in addition to cells housed in the ground controls, vibration controls, and the flight control trays.

The colonies of C. globosum growing in shake cultures with Omeliansky's medium appeared diverse in growth patterns and pigmentation. All colonies grew as small (0.1-3.5 mm) spherical or oval pellets. Dry mycelial weight of each phenotype is presented in Table I. The growth rate variation is evident.

Select flight phenotypes exposed to solar irradiation had either no perithecia or immature perithecial development. Flight control isolates produced normal perithecial development.

Flight phenotypes displayed new agar colony morphology. Some grew as small dense colonies (Cg 6899-1) while others were large, flat colonies (Cg 7102-2). Select ground controls did not appear much different from the Chaetomium flight control in colony morphology. Ground control colonies were distinctive in colony appearance from flight phenotypic colonies.

Phenotypes of C. globosum and the control were cultured in 50 ml of Omeliansky's liquid medium one week for the starch studies. After 168 hours growth, the enzyme production stabilized. A total of 0.3 ml of the liquid medium was pipetted from each flask and analyzed by the RBB starch method. Absorbencies of the various select phenotypes were determined according to described techniques. The number of RBB starch units per 100 ml of the specimen was calculated by the equation: RBB starch units per 100 ml = A_s/A_{cu} x 100. A_s is the average absorbency (sample 1 + 2) at 595 nm of the flask phenotype, and A_{cu} is the average absorbency (sample 1 + 2) at 595 nm of the $CuSO_4$ solution (0.1 M). Three replicates of the RBB starch units were averaged to yield the final results on the Chaetomium control and select phenotype (Table I).

Discussion

The select phenotypes of Chaetomium globosum through postflight experimentation exhibited changes in growth dynamics and microbial physiology. These select phenotypes did respond to the calculated flight parameters to provide a qualitative and quantitative exobiology experiment. Chaetomium globosum flight phenotypes did exhibit variation compared to select phenotypes in the ground control and vibration control.

All of the phenotypes isolated from the Apollo 16 MEED, except Cg 6899-1 and Cg 6904-2, lost pigment forming capability as expressed in the culture grown in the liquid medium. The flight phenotypes were exposed to full light, dark, unvented, and vented, yet they all expressed this pigment alteration greater than control isolates. The synergistic relationship of effects of weightlessness and radiation has been reported (Bender, 1967). The ground control and the vibration control of the select phenotypes expressed no pigmentation alteration when grown in the liquid shake cultures.

The select phenotypes revealed a variation in growth dynamics as expressed in the dry weight study. The flight phenotypes grew at a greater rate as indicated by the dry cell weight compared with the control or ground vibration control. No relationship could be found between the exposed flight phenotypes and the isolates unexposed to UV irradiation with respect to dry weight.

Perithecial development was somewhat lacking in the flight phenotypes. Flight phenotypes displayed different colony morphology as compared to ground control phenotypes.

The Omeliansky's liquid medium provided a good starch source and the source of Ca^{+2} and trace Na^{+} needed for α-amylase production. Starch in the medium was hydrolyzed and utilized by C. globosum.

The flight phenotypes expressed a slightly higher value in RBB starch units than the ground controls and vibration controls. These higher readings, correlated with the dry weight findings and the color alteration demonstrated that select flight phenotypes did undergo cellular change. The RBB starch units expressed among the flight phenotypes do not show any correlation between UV exposed cells and unexposed cells.

Summary

Microbial experimentation using Chaetomium globosum as a space test organism provided a test system to identify effects of space parameters on fungi. Isolated species have been causal agents of damage to space hardware during extended space missions. The microbial damage effects the safety and well being of human astronauts traveling in space.

The current study demonstrated that the MEED flight phenotypes possessed a greater mycelial growth rate than the ground control phenotypes. Flight phenotypes generally lost pigmentation as compared to ground control phenotypes. The MEED flight phenotypes exhibited higher α-amylase production compared to ground control phenotypes. Exposure to space flight environments does induce changes in biological systems as identified in Chaetomium globosum.

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Table I

Chaetomium globosum controls and phenotypes exposed to space irradiation, and postflight observations

Isolate	Ergs/cuvette/ 10 min	Cell housing	Pigmentation	Dry weight	RBB starch units
Cg 7102-2	1.4×10^8 *	dry vented	none	0.39 g	41.95
Cg 6899-1	0	wet flight control	brown	0.47	24.13
Cg 1349-1	1.5×10^5	wet unvented	none	0.42	24.71
Cg 7907-1	1.4×10^8	dry vented	none	0.40	28.73
Cg 6891-1	0	wet flight control	none	0.31	31.60
Cg 6416-1	0	dry vibration control	brown	0.38	18.38
Cg 6898-1	0	dry ground control	brown	0.44	38.50
Cg 1345-2	0	wet ground control	brown	0.30	5.17
Cg 6904-2	1.5×10^7	dry unvented	brown	0.37	27.58
Cg control	0	0	brown	0.33	16.66

* full light of space